AD_____

Award Number: W81XWH-10-1-0246

TITLE: Therapeutic Value of PLK1 Knockdown in Combination with Prostate Cancer Drugs in PIM-1 Overexpressing Prostate Cancer Cells

PRINCIPAL INVESTIGATOR: Meejeon Roh, Ph.D.

CONTRACTING ORGANIZATION: Vanderbilt University
Nashville, TN 37203

REPORT DATE: May 2012

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
May 2012	Annual	15 April 2011 - 14 April 2012

4. TITLE AND SUBTITLE

Therapeutic Value of PLK1 Knockdown in Combination with Prostate Cancer Drugs in PIM-1 Overexpressing Prostate Cancer Cells

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-1-0246

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Meejeon Roh

E-Mail: meejeon.roh@vanderbilt.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The Vanderbilt University

Nashville, TN 37240-0001

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT The treatment options for advanced prostate cancer are limited, thus intensive efforts are ongoing to explore novel targets and strategies for the management of prostate cancer. Our ultimate goal is to identify new target that can specifically sensitize Pim-1 overexpressing prostate cancer cells. Pim-1 is highly overexpressed in prostate cancer and overexpression of Pim-1 leads to genomic instability and docetaxel resistance in prostate epithelial cells. PIM1 synergizes with c-MYC to induce advanced prostate cancer in a kinase-dependent manner. Using a siRNA library screen, we identified Polo-like kinase (PLK1) as a promising target whose knockdown can specifically reduce the cell viability of Pim-1 overexpressing cells. PLK1 is also overexpressed in a wide variety of cancer types including prostate and its expression frequently correlates with poor patient prognosis. PLK1 has been an attractive molecular target for cancer therapy due to its structural hallmarks, its overexpression in various cancer types, and the intrinsic dependence of tumor cells on its activity in mitosis. Silencing of PLK1 has been shown to enhance drug sensitivity in some cancer cells such as pancreatic adenocarcinoma and breast. Our main goal is to test whether depletion of PLK1 will result in synthetic lethality in Pim-1 overexpressing cells and whether depletion of PLK1 will further sensitize Pim-1 overexpressing cells to prostate cancer drugs.

15. SUBJECT TERMS
Plk1, Pim-1, Prostate cancer, Prostate cancer drugs, knockdown

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	9	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

INTRODUCTION

The treatment options for advanced prostate cancer are limited, thus intensive efforts are ongoing to explore novel targets and strategies for the management of prostate cancer. Our ultimate goal is to identify new target that can specifically sensitize Pim-1 overexpressing prostate cancer cells. Pim-1 is highly overexpressed in prostate cancer and overexpression of Pim-1 leads to genomic instability (Dhanasekaran et al., 2001; Roh et al., 2003; Roh et al., 2008) and docetaxel resistance in prostate epithelial cells (Zemskova et al., 2008). PIM1 synergizes with c-MYC to induce advanced prostate cancer in a kinase-dependent manner (Wang et al., 2010). Using a siRNA library screen, we identified Polo-like kinase (PLK1) as a promising target whose knockdown can specifically reduce the cell viability of Pim-1 overexpressing cells. PLK1 is also overexpressed in a wide variety of cancer types including prostate and its expression frequently correlates with poor patient prognosis (Strebhardt and Ullrich, 2006; Weichert et al., 2004). PLK1 has been an attractive molecular target for cancer therapy due to its structural hallmarks, its overexpression in various cancer types, and the intrinsic dependence of tumor cells on its activity in mitosis (Fink et al., 2007). Silencing of PLK1 has been shown to enhance drug sensitivity in some cancer cells such as pancreatic adenocarcinoma and breast (Spankuch et al., 2006; Yu et al., 2008). Our main goal is to test whether depletion of PLK1 will result in synthetic lethality in Pim-1 overexpressing cells and whether depletion of PLK1 will further sensitize Pim-1 overexpressing cells to prostate cancer drugs.

BODY

We will report on studies outlined in Aim 1 and Aim 2 of the proposal which cover Month 13-24.

Aim 1: To examine whether depletion of PLK1 results in synthetic lethality in Pim-1 overexpressing cells.

Task 2: We will examine the mechanistic basis of reduced cell viability of Pim-1 overexppressing prostate cells after PLK1 depletion by checking proliferation, apoptosis, cell cycle distribution and mitotic machinery. ***Task 3:*** We will test whether Pim-1 overexpressing cells lose tumorigenicity after PLK1 depletion by performing *in vitro* soft agar and *in vivo* xenograft assays.

Task, 2 & 3 Results: Previously, we have reported that Plk1 knockdown in Pim-1 overexpresing cells led to significantly reduced cell viability compared to Plk1 knockdown in Neo control cells, consistent with our original siRNA screen result. To understand possible mechanisms that could explain the reason why Pim1 sensitizes cells to the effects of PLK1 inhibition, we focused on MYC and p53. There appears to be a complex relationship between PLK1 and p53. The two proteins have been reported to negatively regulate each other (Ando et al., 2004), and some studies have suggested p53-deficiency makes cells more sensitive to PLK1 inhibition (Guan et al., 2005). We find that PLK1 knockdown increased the levels of p53 in LNCaP and DU145 cells (Fig. 1). A recent study suggested a link between PLK1 and the regulation of MYC protein levels in the G2 phase of the cell cycle (Popov et al., 2010). The ubiquitin ligase β-TrCP was found to ubiquitylate MYC, leadng to increased MYC stability. Phosphorylation of MYC by

PLK1 increases its association with β-TrCP, thereby enhancing MYC stability. We tested this idea by examing MYC protein levels in cells following PLK1 inhibition by shRNA or the potent and specific PLK1 inhibitor BI2536 (Fig. 1). We found that inhibition of PLK1 either by shRNA or BI 2536 in prostate cancer cell lines results in a reduction in MYC protein levels, and an increase in p53 levels (Fig. 1).

Next, we determined if PLK1 depletion will impair the *in vivo* tumorigencity of Pim1-expressing cells. We generated xenografts tumors using LNCaP-Pim1 cells and LNCaP-Neo cells with and without stable shPLK1 expression (Fig. 2). We found that Pim1-expressing LNCaP cells with PLK1 knockdown (Pim1/shPlk1) formed significantly smaller tumors than the LNCaP-Pim1 controls (Pim1) (Fig. 2). By contrast, no differences were observed between LNCaP-Neo and LNCaP-Neo/shPLK1 cells (Fig.2). Thus, our results indicate that Pim1-overexpressing cells are sensitive to Plk1 loss.

AIM 2: To test whether depletion of PLK1 further sensitizes Pim-1 overexpressing cells to prostate cancer drugs.

Task 1. We will test whether loss of PLK1 can synergize with prostate cancer drugs in Pim-1 overexpressing prostate cells.

Task 1 Results: We have reported that loss of Plk1 sensitized with doxorubicin in Pim-1 overexpressing cells in LNCaP cells. Using Plk1 inhibitor, BI 2536, we confirmed that Pim1 overexpressing cells are sensitive to Plk1 inhibition than control neo cells (Fig. 4). Furthermore, BI 2536 and Pim-1 overexpression have synergistic effects on doxorubicin response in LNCaP cells (Fig. 4).

KEY RESEARCH ACCOMPLISHMENTS

- Demonstration that inhibition of PLK1 either by shRNA or Plk1 inhibitor BI2536 in prostate cancer cell lines results in a reduction in MYC protein levels, and an increase in p53 levels.
- Demonstration that PLK1 depletion impairs the *in vivo* tumoriegencity of Pim1-expressing cells.
- Demonstration that Plk1 inhibitor BI 2536 and Pim-1 overexpression have synergistic effects on doxorubicin response in LNCaP cells.

REPORTABLE OUTCOMES

Abstract of manuscript in preparation:

PIM1 kinase is overexpressed in many tumor types including lymphomas and prostate cancer, where it is known to cooperate with the MYC oncogene in promoting tumorigenicity. PIM1 enhances MYC stability and transcriptional activity. Knowledge of the vulnerabilities of Pim1-expressing tumor cells will be of great value in efforts to develop novel anti-cancer therapeutics. Here we have used RNAi screening to identify genes whose depletion is detrimental to Pim1 overexpressing cells. We screened a collection of 570 siRNAs targeting cell cycle, apoptosis,

serine-threonine kinase and tyrosine kinase genes for their effects on the viability of Pim1-expressing RWPE1 prostate epithelial cells. We identified the mitotic regulator polo-like kinase (PLK1) as a gene whose depletion is particularly detrimental to the viability of Pim1-expressing prostate cancer cells in vitro and in vivo. Inhibition of PLK1 by siRNA in several prostate cancer cell lines (as well as xenograft tumors) resulted in a reduction in MYC protein levels. These results suggest that PLK1 inhibition may be particularly effective against PIM1-expressing tumors due, at least in part, to the fact that PLK1 inhibition reduces MYC protein levels.

CONCLUSION

Previously, we have reported that Plk1 knockdown in Pim-1 overexpresing cells led to significantly reduced cell viability compared to Plk1 knockdown in Neo control cells, consistent with our original siRNA screen result. To understand possible mechanisms that could explain the reason why Pim1 sensitizes cells to the effects of PLK1 inhibition, we focused on MYC and p53. We found that inhibition of PLK1 either by shRNA or BI 2536 in prostate cancer cell lines results in a reduction in MYC protein levels, and an increase in p53 levels (Fig. 1). To determine if PLK1 depletion will impair the *in vivo* tumoriegencity of Pim1-expressing cells, we generated xenografts tumors using LNCaP-Pim1 cells and LNCaP-Neo cells with and without stable shPLK1 expression .Our results indicate that PLK1 depletion impairs the *in vivo* tumoriegencity of Pim1-expressing cells (Fig. 2). We have also found that depletion of Plk1 sensitized with doxorubicin in Pim-1 overexpressing cells in LNCaP cells using Plk1 inhibitor, BI 2536 (Fig. 4). Going on, tumorigenicity of Plk1 loss in combination with prostate drugs in Pim-1 overexpressing cells will be tested. Then, the underlying mechanism for the synthetic lethality between Plk1 depletion and Pim 1 overexpression will be explored.

REFERENCES

Ando, K., Ozaki, T., Yamamoto, H., Furuya, K., Hosoda, M., Hayashi, S., Fukuzawa, M., and Nakagawara, A. (2004). Polo-like kinase 1 (Plk1) inhibits p53 function by physical interaction and phosphorylation. J Biol Chem *279*, 25549-25561.

Dhanasekaran, S. M., Barrette, T. R., Ghosh, D., Shah, R., Varambally, S., Kurachi, K., Pienta, K. J., Rubin, M. A., and Chinnaiyan, A. M. (2001). Delineation of prognostic biomarkers in prostate cancer. Nature *412*, 822-826.

Fink, J., Sanders, K., Rippl, A., Finkernagel, S., Beckers, T. L., and Schmidt, M. (2007). Cell type-- dependent effects of Polo-like kinase 1 inhibition compared with targeted polo box interference in cancer cell lines. Mol Cancer Ther *6*, 3189-3197.

Guan, R., Tapang, P., Leverson, J. D., Albert, D., Giranda, V. L., and Luo, Y. (2005). Small interfering RNA-mediated Polo-like kinase 1 depletion preferentially reduces the survival of p53-defective, oncogenic transformed cells and inhibits tumor growth in animals. Cancer Res *65*, 2698-2704.

Popov, N., Schulein, C., Jaenicke, L. A., and Eilers, M. (2010). Ubiquitylation of the amino terminus of Myc by SCF(beta-TrCP) antagonizes SCF(Fbw7)-mediated turnover. Nat Cell Biol *12*, 973-981.

Roh, M., Franco, O. E., Hayward, S. W., van der Meer, R., and Abdulkadir, S. A. (2008). A role for polyploidy in the tumorigenicity of Pim-1-expressing human prostate and mammary epithelial cells. PLoS ONE *3*, e2572.

Roh, M., Gary, B., Song, C., Said-Al-Naief, N., Tousson, A., Kraft, A., Eltoum, I. E., and Abdulkadir, S. A. (2003). Overexpression of the oncogenic kinase Pim-1 leads to genomic instability. Cancer Res *63*, 8079-8084.

Spankuch, B., Heim, S., Kurunci-Csacsko, E., Lindenau, C., Yuan, J., Kaufmann, M., and Strebhardt, K. (2006). Down-regulation of Polo-like kinase 1 elevates drug sensitivity of breast cancer cells in vitro and in vivo. Cancer Res *66*, 5836-5846.
Strebhardt, K., and Ullrich, A. (2006). Targeting polo-like kinase 1 for cancer therapy. Nat Rev Cancer *6*, 321-330.

Wang, J., Kim, J., Roh, M., Franco, O. E., Hayward, S. W., Wills, M. L., and Abdulkadir, S. A. (2010). Pim1 kinase synergizes with c-MYC to induce advanced prostate carcinoma. Oncogene *29*, 2477-2487.

Weichert, W., Schmidt, M., Gekeler, V., Denkert, C., Stephan, C., Jung, K., Loening, S., Dietel, M., and Kristiansen, G. (2004). Polo-like kinase 1 is overexpressed in prostate cancer and linked to higher tumor grades. Prostate *60*, 240-245.

Yu, C., Zhang, X., Sun, G., Guo, X., Li, H., You, Y., Jacobs, J. L., Gardner, K., Yuan, D., Xu, Z., *et al.* (2008). RNA interference-mediated silencing of the polo-like kinase 1 gene enhances chemosensitivity to gemcitabine in pancreatic adenocarcinoma cells. J Cell Mol Med *12*, 2334-2349.

Zemskova, M., Sahakian, E., Bashkirova, S., and Lilly, M. (2008). The PIM1 kinase is a critical component of a survival pathway activated by docetaxel and promotes survival of docetaxel-treated prostate cancer cells. J Biol Chem *283*, 20635-20644.

APPENDICES

None

SUPPORTING DATA

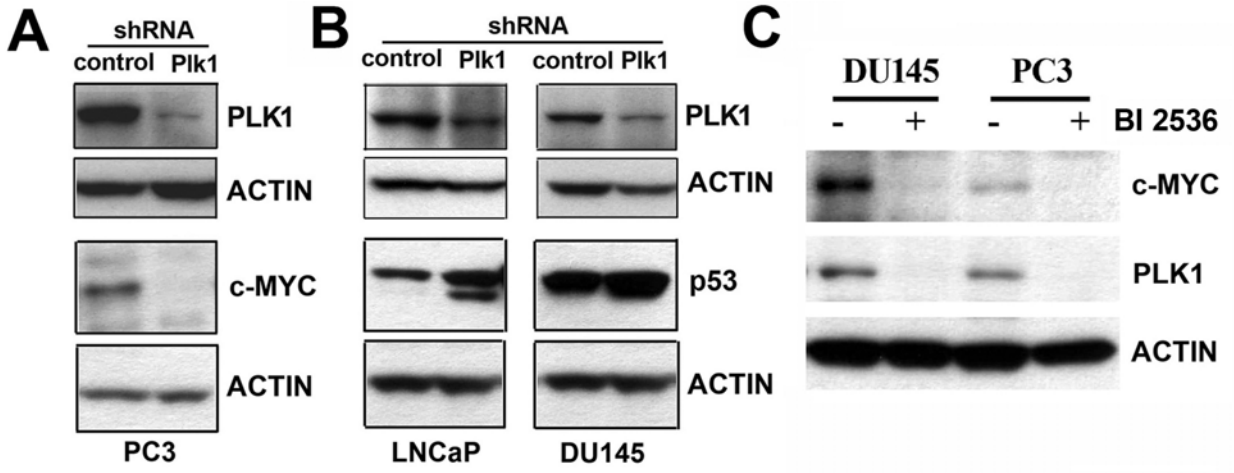

Fig. 1. Effects of Plk1 depletion on Myc and p53 level. Inhibition of PLK1 by shRNA in prostate cancer cell lines results in a reduction in MYC protein levels (A), whereas p53 protein levels were increased in Plk1 knock downed cells (B). A: PC 3 cells. B: LNCaP and DU145 cells. Decreased levels of MYC protein was also confirmed using Plk1 inhibitor, BI 2536 (C).

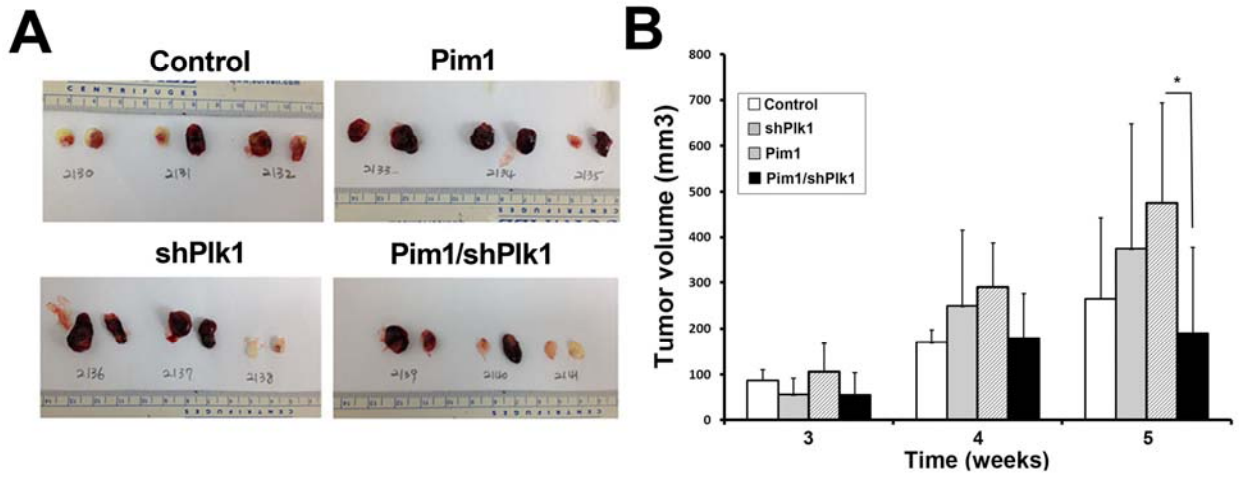

Fig. 2. Knockdown of Plk1 in Pim1 overexpressing LNCaP cells led to the reduced tumor size in nude mouse. LNCaP-Neo control (control), -Pim1 overexpressing cells (Pim1), Plk1 knock-downed control cells (control/shPlk1), and Plk1 knock-downed Pim1- overexpressing cells (Pim1/shPlk1) were inoculated into nude mice. 6 weeks after inoculation, the mice were sacrificed for analysis. (A) Gross images of grafts. (B) Mean tumor volumes of each group after 6 weeks inoculation. 10^7 cells per site were used.

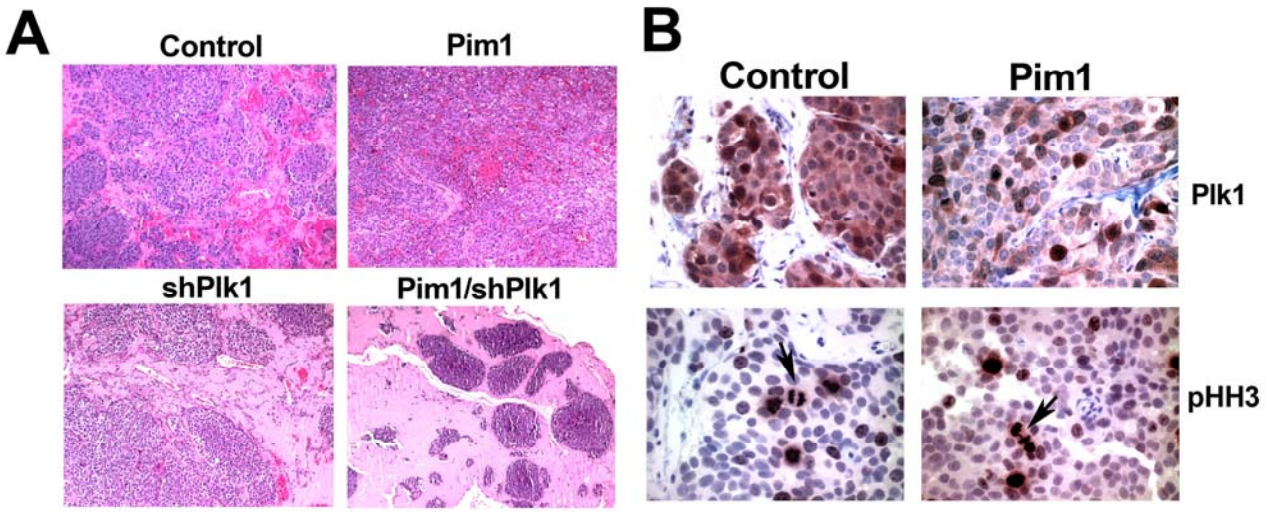

Fig. 3. Pim1-expressing prostate cancer cells are sensitive to PLK1 depletion in vivo. (A) Representative H& E images of each group are shown. Notice that Pim1 cells formed big tumors, whereas Pim1/shPlk1 cells formed small, isolated form of tumor. x10. (B) PLK1 and phospho histone H3 immunostaining in control and Pim1 cells. x 40.

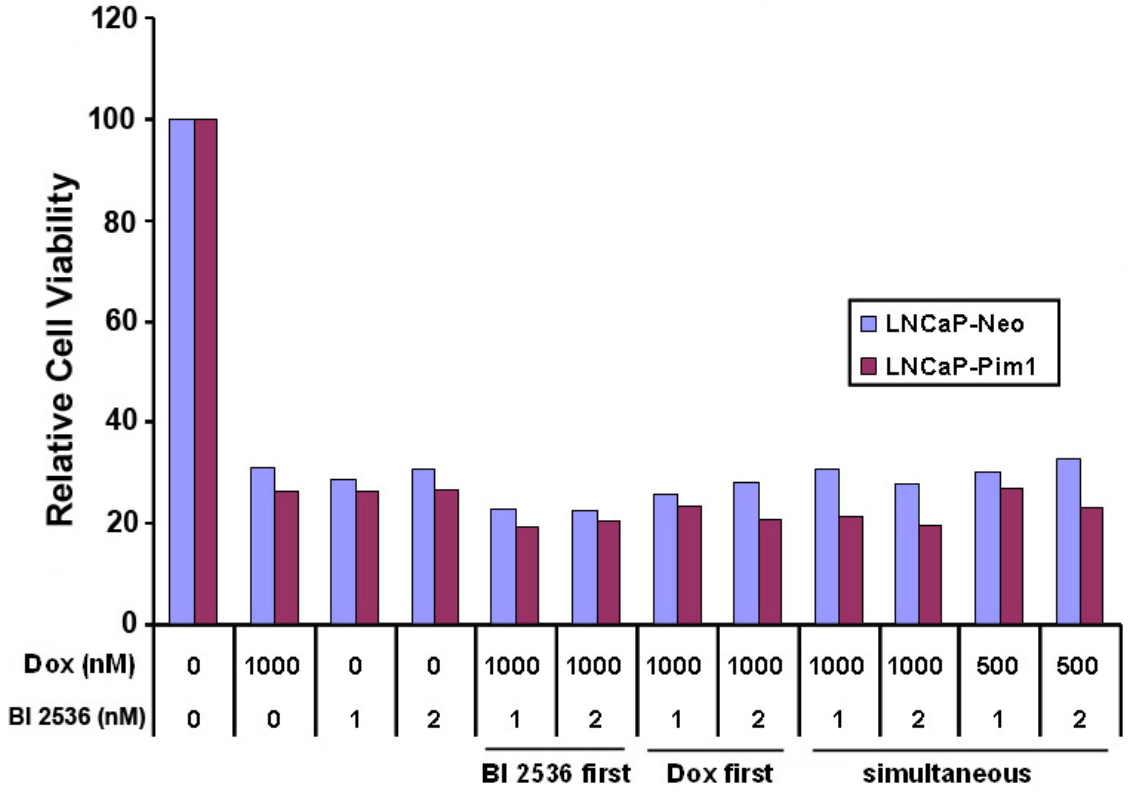

Fig. 4. Effects of BI 2536 on Doxorubicin in LNCaP cells. Pim1 overexpressing cells were more sensitive to drug treatment than Neo cells. LNCaP-Neo and Pim1 overexpressing cells were plated on 96 wells, treated with either doxorubin (Dox) alone, BI2536 alone, or together on following day and the cell viability was determined 2 days after drug treatment. For double treatment, drugs were treated separately (either BI2536 or doxorubicin first) or simultaneously. In all cases, there is a trend that Pim1 overexpressing cells are more sensitive to drug treatment than Neo control cells. Cell viability was measure using CellTiter-Glo Luminescence Cell viability assay.

.